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Molecular Phylogeny of the Genus *Gloydius* (Serpentes: Crotalinae)

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Abstract Based on two mitochondrial genes (cyt b, ND4) and one nuclear gene (c-mos), we explored the relationships within the Asian pit viper genus *Gloydius*. In total, 23 samples representing 10 species were analyzed. All phylogenetic analyses support a monophyletic *Gloydius* with two major clades, one comprising *G. brevicaudus, G. blomhoffii*, and *G. ussuriensis* with the sister clade consisting of *G. intermedius, G. saxatilis, G. halys* and *G. shedaoensis*. The relationships among the three montane species *G. strauchi*, *G. qinlingensis* and *G. liupanensis*, as well as the two monophyletic groups, are unstable, and discussed. Divergence date estimation indicates that *Gloydius* lineage formed 15 Ma and diversification of the genus occurred at 9.89 Ma. Issues regarding the taxonomy of this genus are discussed where necessary.

Keywords molecular phylogenetics, venomous snake, pit viper, mtDNA, nDNA

1. Introduction

The genus *Gloydius* (Serpentes: Crotalinae) is a venomous group of snakes distributed in many places of Asia, including China, Korea, Japan, Russia and Nepal (David and Ineich, 1999; Gumprecht *et al*., 2004), and is diverse in ecology (e. g., montane or lowland, and arboreal or terrestrial) and external morphology (Zhao, 2006). Previous research has focused on the taxonomy (Guo *et al*., 1999), ecology (Shine *et al*., 2002) and evolution (Wüster *et al*., 2008; Ding *et al*., 2011) of this taxon.

Guo *et al*. (1998) reviewed the systematics of this genus. On the basis of comparative skull morphology, Guo *et al*. (1999) discussed the status and validity of its several species. A phylogenetic hypothesis based on morphological characteristics suggested that *Gloydius* could be divided into three groups: *brevicaudus* group, including *G. brevicaudus* and *G. ussuriensis*; *intermedius* group, including *G. intermedius, G. saxatilis* and *G. shedaoensis*; and *strauchi* group, including *G. strauchi, G. liupanensis* and *G. qinlingensis* (Guo *et al*., 2002). However, other authors have proposed different taxonomic arrangements (Zhao, 1979, 1980; Shen *et al*., 1999; Zhou *et al*., 2000, 2001; Li, 2007, 2009). Taxonomic arrangement of the species within *Gloydius* has varied greatly in the literature (Isogawa, 1997; Li, 1999; David and Ineich, 1999; Gumprecht *et al*., 2004; Zhao, 2006; Jiang and Zhao, 2009). Here we explore the systematics and interspecific relationships of the genus *Gloydius* using both mtDNA and nDNA loci, focusing on the species that occur in China.

2. Material and Methods

2.1 Data collection Total genomic DNA was extracted from liver, muscle or blood from 19 samples (Table 1)

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Table 1 Samples used in this study.

Taxon	Voucher No.	Locality	GenBank accession		
			cyt b	ND4	c-mos
Gloydius brevicaudus	GP52	Huangshan, Anhui, China	JQ687486	JQ687467	JQ687505
G. brevicaudus	GP628	Kuandian, Liaoning, China	JQ687495	JQ687476	JQ687514
G. brevicaudus	GP1027	China	JQ687496	JQ687477	JQ687515
G. brevicaudus	GP1099	Lüshun, Liaonling, China	JQ687497	JQ687478	JQ687516
G. brevicaudus	GP1480	Xinglong, Hebei, China	JQ687504	JQ687485	JQ687523
G. brevicaudus	AM B525		AY352752	AY352815	
G. ussuriensis	GP1326	Ji'an, Jilin, China	JQ687501	JQ687482	JQ687520
G. ussuriensis	GP1331	Tonghua, Jilin, China	JQ687503	JQ687484	JQ687522
G. qinlingensis	GP197	Zhouzhi, Shaanxi, China	JQ687490	JQ687471	JQ687509
G. liupanensis	GP198	Ningxia, China	JQ687491	JQ687472	JQ687510
G. liupanensis	GP206	Ningxia, China	JQ687492	JQ687473	JQ687511
G. liupanensis	GP215	Ningxia, China	JQ687493	JQ687474	JQ68751
G. strauchi	GP175	Heishui, Sichuan, China	JQ687487	JQ687468	JQ687506
G. intermedius	GP465	Xinjiang, China	JQ687494	JQ687475	JQ687513
G. intermedius	GP188	Xinjiang, China	JQ687488	JQ687469	JQ687507
G. saxatilis	GP191	Hengren, Liaonling, China	JQ687489	JQ687470	JQ687508
G. saxatilis	GP1328	Ji'an, Jilin, China	JQ687502	JQ687483	JQ687521
G. shedaoensis	GP1100	Lüshun, Liaoning, China	JQ687498	JQ687479	JQ687517
G. shedaoensis	GP1110	Lüshun, Liaoning, China	JQ687499	JQ687480	JQ687518
G. shedaoensis	GP1116	Lüshun, Liaoning, China	JQ687500	JQ687481	JQ687519
G. shedaoensis	ROM20468	Lüshun, Liaoning, China	AY223566	AY223623	
G. blomhoffi	-		AY352751	AY352814	
G. halys	-		AY223564	AY223621	
Prortobothrops mangshanensis	GP17	Yizhang, Hunan, China	HM567537	HM567469	JQ687524
Bothriechis schlegelii	-		AF292573	U41874	
Viridovipera stejnegeri	-		EF597524	EF597529	
Porthidium ophryomegas	-		AY223580	U41888	
P. nasutum	-		AY223579	U41887	
Naja nigricollis	-		EU624300	AY713377	
N. naja	-		EU624299	AY713378	
Hemorrhois algirus	-		AY486911	AY487037	
H. nummifer	-		AY376742	AY487049	
Acrochordus granulatus			NC007400	NC007400	

GP: Guo Peng, for author's own catalogue numbers.

representing eight species using the phenol/chloroform method (Sambrook and Russell, 2002). Complete gene sequences for cytochrome *b* (cyt b), partial gene sequences of NADH dehydrogenase subunit 4 (ND4) and the nuclear gene c-mos were amplified *via* polymerase chain reaction (PCR) using the following primers: L14910/H16064 (Burbrink *et al*., 2000), ND4/Leu (Arevalo *et al*., 1994) and S77/S78 (Lawson *et al*., 2005), respectively. Protocol for amplification followed Burbrink *et al*. (2000), Arevalo *et al*. (1994) and Lawson *et al*. (2005). Double-stranded products were sequenced using an ABI 3730 Genetic Analyzer (Applied Biosystems) following manufacturer's protocols. All mtDNA sequences were aligned using ClustalW in Mega 4.0 with default settings (Tamura *et al*., 2008) and checked by eye to ensure pseudogenes were not amplified (Zhang and Hewitt, 1996).

2.2 Phylogenetic reconstruction Generated sequence data was analyzed with four additional samples representing *G. blomhoffii*, *G. brevicaudus*, *G. shedaoensis* and *G. halys*, whose sequences are from GenBank. Three taxa (*Protobothrops mangshanensis, Bothriechis schlegelii, Viridovipera stejnegeri*; Table 1) were chosen as outgroups on the basis of previous work (Malhotra and Thorpe, 2004). Phylogenetic analyses were performed using three different methods: Bayesian inference (BI), maximum parsimony (MP) and maximum likelihood (ML). For BI, sequences were divided into seven partitions: mitochondrial protein-coding genes were partitioned into 1st, 2nd and 3rd codon positions and c-mos. For each partition the best-fit model of nucleotide substitution was determined using AIC in MrModeltest 2.3 (Ronquist and Huelsenbeck, 2003; Nylander, 2004). Three independent runs initiated from random trees were conducted in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). All searches consisted of four Markov chains (three heated chains and a single cold chain), estimated for 40 million generations and sampled every 3 000 generations with 20% of the initial samples discarded as burn-in. Substitution parameters were unlinked and rates were allowed to vary across partitions. Stationarity was confirmed in the program Tracer v1.4 (Rambaut and Drummond, 2007). The resultant trees were combined as a 50% majority-rule consensus tree to calculate posterior probabilities (PP) for each node. The ML tree and associated support indices were obtained under the same partition strategy executed in RAxML (Stamatakis *et al.*, 2008). MP trees were generated using PAUP* 4.0b10 (Swofford, 2003) from equally weighted characters with a heuristic search using 1 000 random sequence addition replicates and tree bisection–reconnection (TBR) branch swapping. Support values (BS) for clades were calculated from 1 000 bootstrap pseudoreplicates (Felsenstein, 1985). Average divergence estimation between species was calculated from two mitochondrial genes using Mega 4.0 (Tamura *et al.*, 2008).

2.3 Divergence time estimation The program BEAST v 1.5.1 (Drummond and Rambaut, 2007) was used to estimate the date of origin of *Gloydius* under the assumption of a relaxed molecular clock using BI (Drummond *et al.*, 2006). The conditions of this analysis were identical to those of the Bayesian phylogenetic inference above. Rates and times were estimated under the uncorrelated lognormal tree prior, with a birth-death prior on speciation and Jeffrey's priors on the substitution model parameters. For this analysis, only two mtDNA sequences were used, and several additional species were included and their sequences were retrieved from GenBank (*Porthidium ophryomegas*, *P. nasutum*, *Naja nigricollis*, *N. naja*, *Hemorrhois algirus*, *H. nummifer*, *Acrochordus granulatus*; Table 1). Of them, *A. granulatus* was considered as outgroup based on previous work (Pyron *et al.*, 2011)

Three calibrations were used to date the tree: 1) The divergence between *P. ophryomegas* and *P. nasutum* was modeled with a normal distribution, with a mean of 3.5 Ma and a standard deviation of 0.51 (Wüster *et al.*, 2002); 2) the split between *N. nigricollis* and *N. naja* was given a lognormal prior with a 16 Ma zero offset, lognormal mean of 1 and a lognormal standard deviation of 1 (Szyndlar and Rage, 1990; Wüster *et al.*, 2007); and 3) the divergence between *H. algirus* and *H. nummifer* was calibrated with a normal prior having a mean of 18 Ma (Nagy *et al.*, 2003) and a standard deviation of 2.04. We assumed convergence when the effective sample size (ESS) of the posterior probability distribution of all parameters was > 200 (Drummond *et al.*, 2006), calculated in Tracer v1.4 (Rambaut and Drummond, 2007).

3. Results

3.1 Sequence data The final alignment of three gene fragments consisted of a total of 2 282 base pairs: 1 067 from cyt b, 665 from ND4 and 550 from c-mos. The concatenated dataset contained 619 variable sites, of which 438 were phylogenetically informative under MP criteria. No deletions, insertions or stop codons were detected, indicating that the unintentional amplification of pseudogenes was unlikely (Zhang and Hewitt, 1996). Average divergence distances based on the mtDNA loci between species are given in Table 3. Novel sequences were deposited in GenBank (Table 1).

3.2 Phylogenetic analyses The best-fit model for each partition is listed in Table 2. In the BI tree, all representatives of *Gloydius* were shown to be monophyletic with strong support (100%; Figure 1). Within *Gloydius*, *G. brevicaudus*, *G. ussuriensis* and *G. blomhoffi* formed a highly supported monophyletic group, of which *G. ussuriensis* showed a sister relationship with *G. blomhoffi*, and then both were sisterly related to *G. brevicaudus*. *G. intermedius*, *G. halys*, *G. saxatilis*, *G. shedaoensis*, *G. strauchi* and *G. liupanensis* formed another monophyletic group with strong support (99%). Within this clade, *G. intermedius*, *G. halys*, *G. saxatilis*, *G. shedaoensis* formed a subclade with high support value (100%), and *G. saxatilis* showed a sister relationship with *G. shedaoensis*. Interestingly, the two samples representing *G. intermedius* are not recovered as sister taxa. The MP and ML trees topologies are generally consistent with the BI tree. The main differences are indicated by the positions of *G. qinlingensis*, *G. strauchi* and *G. liupanensis* (Figure 1).

Table 2 Results of AIC model selection conducted in MrModeltest for different partitions of the dataset.

Partition	AIC model
cyt-b, position 1	HKY+I+G
cyt-b, position 2	GTR+I
cyt-b, position 3	GTR+G
cyt-b	GTR+I+G
ND4, position 1	HKY
ND4, position 2	HKY
ND4, position 3	GTR+G
ND4	HKY+G
c-mos	HKY

3.3 Divergence date estimation Divergence dating indicates that the origin of *Gloydius* occurred at about 15

Ma. The diversification within the genus began at about 9.89 Ma (95% HPD: 5.17–16.79 Ma) (Figure 2).

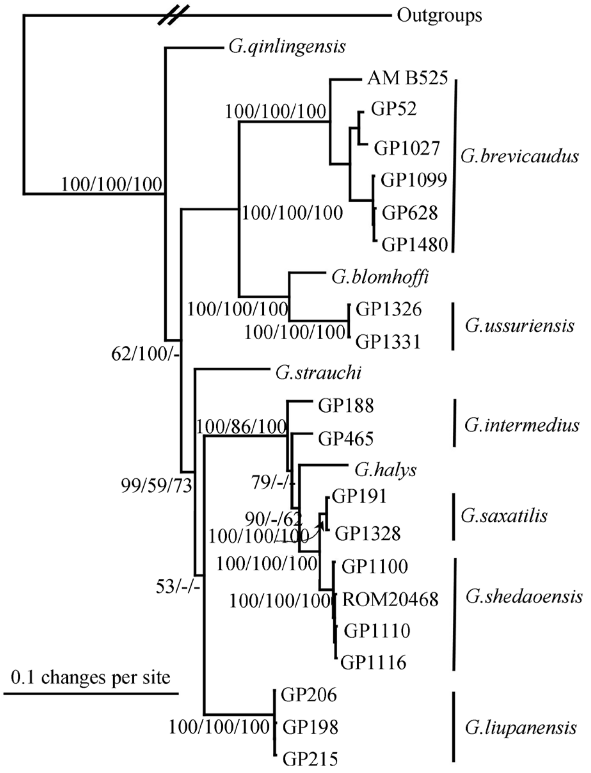

Figure 1 Bayesian 50% majority-rule consensus phylogram inferred using 7-partiton model from nuclear and mtDNA data in MrBayes. The values assigned to the internodes indicate support for BI/MP/ML. A node with support value < 50% is indicated by "-".

4. Discussion

Phylogeny reconstruction using three different methods generally produced congruent results, in which all the representatives of *Gloydius* analyzed formed a well supported group (Figure 1). The interspecific relationships presented here are consistent with those of previous results based on morphological comparisons to some extent (Guo *et al.*, 2002). The monophyly including species *G. brevicaudus*, *G. ussuriensis* and *G. blomhoffi* generally exhibit 21 rows of dorsal scales at mid-body and have 4 palatine teeth, while those including *G. intermedius*, *G. halys*, *G. saxatilis* and *G. shedaoensis* possess 23 rows of dorsal scales at mid-body and have 3 palatine teeth. However, another species group defined previously, which includes the three montane species (*G. strauchi*, *G. qinlingensis*, *G. liupanensis*), was not recovered in our analysis (Figure 1).

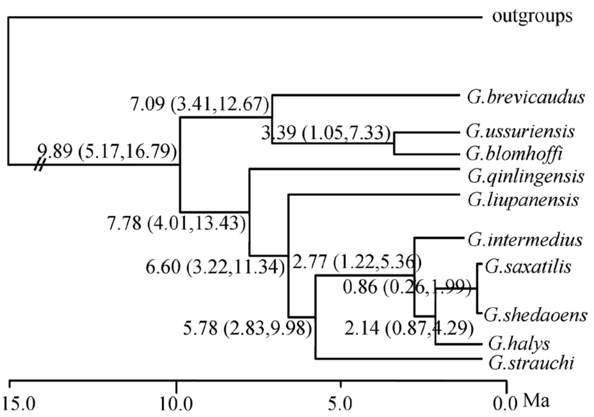

Figure 2 Bayesian estimates of divergence time (Ma) with 95% HPD of the genus *Gloydius* computed using BEAST (Drummond and Rambaut, 2007).

Of the Chinese *Gloydius* species, controversy has existed regarding the systematic position of *G. qinlingensis* and *G. liupanensis* since they were initially described (Zhao *et al.,* 1998; Zhao and Yang, 1997; Guo *et al.*, 1999; Gumprecht *et al.*, 2004; Wang and Zhao, 2006). Zhao *et al.* (1998) synonymized both with *G. strauchi*. Based on comparisons of skull characteristics, Guo *et al.* (1999) recognized *G. qinlingensis* to be a valid species. On the basis of external morphology, Wang

Table 3 Uncorrected *P*-distance between species of *Gloydius* for cyt *b* (above the diagonal) and ND4 (below the diagonal) sequences.

Species	*G. brevicaudus*	*G. strauchi*	*G. intermedius* 1	*G. saxatilis*	*G. qinlingensis*	*G. liupanensis*	*G. intermedius* 2	*G. shedaoensis*	*G. ussuriensis*	*G. halys*	*G. blomhoffi*
G. brevicaudus		0.11	0.12	0.13	0.12	0.12	0.12	0.14	0.10	0.14	0.09
G. strauchi	0.13		0.09	0.09	0.06	0.08	0.09	0.10	0.10	0.09	0.10
G. intermedius 1	0.15	0.11		0.04	0.09	0.10	0.03	0.05	0.12	0.06	0.11
G. saxatilis	0.14	0.11	0.03		0.09	0.11	0.03	0.02	0.12	0.05	0.12
G. qinlingensis	0.12	0.09	0.11	0.11		0.08	0.09	0.10	0.11	0.09	0.10
G. liupanensis	0.12	0.08	0.10	0.09	0.07		0.10	0.12	0.14	0.11	0.13
G. intermedius 2	0.14	0.10	0.03	0.03	0.10	0.10		0.04	0.12	0.05	0.10
G. shedaoensis	0.15	0.12	0.04	0.01	0.12	0.10	0.04		0.13	0.06	0.12
G. ussuriensis	0.11	0.12	0.12	0.11	0.12	0.12	0.13	0.11		0.11	0.06
G. halys	0.14	0.10	0.04	0.03	0.11	0.09	0.04	0.04	0.12		0.11
G. blomhoffi	0.10	0.10	0.12	0.12	0.11	0.10	0.13	0.13	0.05	0.12	

and Zhao (2006) suggested that *G. qinlingensis* was a subspecies of *G. strauchi*, and *G. liupanensis* was the most northern population of *G. strauchi*. Unfortunately, the molecular phylogeny presented here did not resolve the relationships among *G. strauchi*, *G. qinlingensis* and *G. liupanensis*. The genetic distances (uncorrected *P*-distance) among these three species are comparable to those observed between other *Gloydius* species compared (Table 3). Considering both the morphological and molecular data, we suggest that both *G. qinlingensis* and *G. liupanensis* are valid species. However, the unsolved systematic relationships need to be further examined with both more intensive specimen sampling and the application of multiple unlinked loci.

Divergence date estimation indicates that *Gloydius* originated in the mid-Miocene (about 15 Ma) and that species differentiation began in the late Miocene (about 9.89 Ma), which is consistent with previous analyses (Guo *et al.*, 2002). The Qinghai-Xizang Plateau began its slow uplift during the Miocene (about 25–10 Ma) with a rapid uplift at about 3.4 Ma in the mid-Pliocene (Sun, 1997). It is notable that the divergence of the genus *Gloydius* occurred during the uplifting of the Qinghai-Xizang Plateau. The uplifting of the Plateau, which would produce physical barriers for genetic exchange, coupling with climate fluctuation and glacial cycles, would lead to population extraction and expansion, possibly being responsible for the speciation of each group.

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References

Arévalo E., Davis S. K., Site W. J. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Syst Biol, 43: 387–418

Burbrink F. T., Lawson R., Slowinski J. B. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): A critique of the subspecies concept. Evolution, 54: 2107–2118

David P., Ineich I. 1999. Les serpents venimeux du monde: Systématique et repartition. Dumerilia, 3: 3–499

Ding L., Gan X., He S., Zhao E. M. 2011. A phylogeographic, demographic and historical analysis of the short-tailed pitviper *(Gloydius brevicaudus)*: Evidence for early divergence and late expansion during the Pleistocene. Mol Ecol, 20: 1905–1922

Drummond A., Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol, 7: 214–225

Drummond A., Simon Y., Phillips M., Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol, 5: 699–710

Felsenstein J. 1985. Confidence limits on phylogenies: A method using the bootstrap. Evolution, 39: 781–791

Gumprecht A., Tillack F., Orlov N. L., Captain A. 2004. Asian Pitvipers. Berlin: Geitje Books, 368 pp

Guo P., Zhang F. J. 1998. The studies on the classification of the genus *Gloydius* from China. Sichuan J Zool, 17(4): 166–169 (In Chinese)

Guo P., Zhang F. J., Chen Y. Y. 1999. Comparative studies on the skull morphology of Chinese species of *Agkistrodon* and *Deinagkistrodon* with discussion on their classification (Serpentes: Crotalinae). Zool Res, 20(6): 415–420 (In Chinese)

Guo P., Zhang J. F. 2002. Phylogenetic and biogeographic studies on *Deinagkistrodon* and *Gloydius* in China. J Sichuan Univ (Nat Sci), 39(2): 378–381 (In Chinese)

Huelsenbeck J. P., Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenentic trees. Bioinformatics, 17: 754–755

Isogawa Q. 1997. Classification problems of *Agkistrodon* (Viperidae: Crotalinae) in Old World. Mamm Sci, 37(1): 47–53 (In Japanese with English abstract)

Jiang F., Zhao E. M. 2009. *Gloydius lijianlii*, a new species from the northern coastal islands along Shandong Peninsula (Reptilia, Squamata, Viperidae). Acta Zoota Sin, 34(3): 642–646.

Lawson R., Slowinski J. B., Crother B. I., Burbrink F. T. 2005. Phylogeny of the Colubroidea (Serpentes): New evidence form mitochondrial and nuclear genes. Mol Phylogenet Evol, 37: 581–601

Li J. L. 1999. Infraspecific classification of two species of *Gloydius* (Serpentes: Crotalinae). Acta Zoota Sin, 24(4): 454–460 (In Chinese)

Li L. 2007. Phylogeny of partial *Gloydius* based on cytochrome *b* gene sequence. Master Thesis, Shaanxi Norm Univ, 35 pp (In Chinese)

Li X. P. 2009. System development and geographical evolution of *Gloydius* in Qinling area. Master Thesis, Shaanxi Norm Univ, 59 pp (In Chinese)

Malhotra A., Thorpe R. S. 2004. A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pitvipers. Mol Phylogenet Evol, 32: 83–100

Nagy Z. T., Joger U., Wink M., Glaw F., Vences M. 2003. Multiple colonization of Madagascar and Socotra by colubrid snakes: Evidence from nuclear and mitochondrial gene phylogenies. Proc R Soc Lond B, 270: 2613–2621

Nylander J. A. A. 2004. MrModeltest v.2 Program, distributed by the author. Evolutionary Biology Centre, Uppsala Univ

Olivier L., Xavier B., Richard S. 2002. Taxonomic chauvinism. Trends Ecol Evol, 17: 1–3

Pyron R. A., Burbrink F. T., Colli G. R., de Oca A. N. M., Vitt L. J., Kuczynski C. A., Wiens J. J. 2011. The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. Mol Phylogenet Evol, 58: 329–342

Rambaut A., Drummond A. J. 2007. Tracer v1.4. Available from http://evolve.zoo.ox.ac.uk

Ronquist F., Huelsenbeck J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Biol Appl Note, 19: 1572–1574

Sambrook J., Russell D. W. 2002. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, New York: Cold Spring Harbor Lab Press, 1950 pp

Shen Y., Zhou K. Y., Wang Y. Q. 1999. RAPD analysis of pitvipers of the genus *Agkistrodon* in China. Acta Zool Sin, 45(1): 40–48 (In Chinese)

Shine R., Sun L. X., Kearney M., Fitzgeral M. 2002. Thermal correlates of foraging-site selection by Chinese pit-vipers (*Gloydius shedaoensis*, Viperidae). J Therm Biol, 27: 405–412

Stamatakis A., Hoover P., Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst Biol, 57: 758–771

Sun H. L. 1997. Research of the formation, enviroment change on Qinghai-Xizang (Tibetan) Plateau. Changsha, China: Hunan Science & Technology Press (In Chinese)

Swofford D. L. 2003. PAUP*: Phylogenetic analysis using parsimony, version 4.0b10, and other methods, version 4.0. Sunderland, Massachusetts: Sinauer Associates

Szyndlar Z., Rage J. C. 1990. West Palearctic cobras of the genus *Naja* (Serpentes: Elapidae): Interrelationships among extinct and extant species. Amphibia-Reptilia, 11: 385–400

Tamura K., Dudley J., Nei M., Kumar S. 2008. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol, 24: 1596–1599

Wang X. J., Zhao E. M. 2006. A preliminary taxonomic status of *Gloydius qinlingensis* and *Gloydius liupanensis*. Sichuan J Zool, 25(2): 210–213

Wüster W., Crookes S., Ineich I., Mané Y., Pook C. E., Trape J. F., Broadley D. G. 2007. The phylogeny of cobras inferred from mitochondrial DNA sequences: Evolution of venom spitting and the phylogeography of the African spitting cobras (Serpentes: Elapidae: *Naja nigricollis* complex). Mol Phylogenet Evol, 45: 437–453

Wüster W., Peppin L., Pook C. E., Walker D. E. 2008. A nesting of vipers: Phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). Mol Phylogenet Evol, 49: 445–459

Wüster W., Salomão M. G., Quijada-Mascareñas J. A., Thorpe R. S. 2002. Origin and evolution of the South American pitviper fauna: Evidence from mitochondrial DNA sequence analysis. In Schuett G. W., Höggren M., Douglas M. E., Greene H. W. (Eds.), Biology of the Vipers. Eagle Mountain: Eagle Mountain Publishing, 111–128

Zhang D. X., Hewitt G. M. 1996. Nuclear integrations: Challenges for mitochondrial DNA markers. Trends Ecol Evol, 11: 247–251

Zhao E M. 1979. A new *Agkistrodon* from Shedao (Snake Island), Liaoning. Acta Herpetol Sin, 1(1): 4–6 (In Chinese)

Zhao E M. 1980. Taxonomic study of Pit-viper of Shedao (Snake Island), by morphological and experimental methods and a preliminary discussion on the origin of Snake-Island Pit-viper on Shedao. Acta Herpetol Sin, 4(1): 1–16 (In Chinese)

Zhao E. M. 2006. Snakes in China, Vol. 1. Hefei, China: Anhui Science and Technology Publishing House (In Chinese)

Zhao E. M., Yang D. T. 1997. Amphibians and Reptiles of the Hengduan Mountains Region. Beijing: Science Press

Zhao E. M., Huang M. H., Zong Y. 1998. Fauna Sinica, Reptilia 3: Squamata Serpents. Beijing: Science Press (In Chinese)

Zhou J. L., Yao Y. G., Huang M. H., Yang D. T., Lu S. Q., Zhang Y. P. 2000. Phylogenetic relationships among viperidae, crotalinae based on mitoehondrial 12s rRNA sequence variations. Acta Gene Sin, 27(4): 283–289 (In Chinese)

Zhou J. L., Zhang Y. P., Huang H. M., Chen Y. J., Chen X. Q., Yao G. D. 2001. Phylogenetic relationships among crotalinaes based on mitochondrial cytochrome *b* gene sequence variations. Acta Zool Sin, 47(4): 361–366 (In Chinese)